5 METHOD AND APPARATUS FOR EXAMINING FLUIDS OF BIOLOGICAL ORIGIN

a. BACKGROUND

The present invention relates to a method for examining a fluid according to the preamble of claim 1. It relates further to devices for executing the method.

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10 Clinical laboratory tests are routinely performed on the serum or plasma of whole blood. The tests commonly employ a series of reactions which terminate after the generation of chromophores which facilitate detection by spectroscopic measurements. The accuracy of most spectroscopic tests is
15 affected to some extent by in vitro interferences. In vitro interferences arise from the fact that biochemical analysis are performed in the complex matrices that make up biological fluids, e.g. serum, plasma or urine. These fluids contain numerous compounds that either have chemical groups
20 that can react with the test reagents or can have the physical or spectral properties of the target analyte. Further, the chemical composition of body fluids can vary with the nature and the extent of disease processes. In vitro interferences can be classified into two classes:
25 spectral and chemical interference. The most commonly observed interferences are hemolysis, icteria, and lipemia. Some 30 % of samples obtained from clinic or hospitalized patients are hemolyzed, icteric, or lipemic. Main reasons for hemolysis are unskilled blood taking or sample
30 preparation, for icteria the jaunice disease, and for lipemia fat nutrition before blood taking.

The goal of sample quality monitoring is the determination of the interfering substances hemoglobin, bilirubin, and lipid prior to conducting fully automated clinical
35 laboratory tests in order to provide meaningful and accurate test results. If a sample is sufficiently contaminated with

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5 interference substances, the test may either not be
   conducted or the test result may be flagged to indicate that
   it is not reliable. Particularly, such a test is desirable
   in connection with the use of clinical-chemical analyzers
   which perform most of the analysis of a sample fully
10 automatically and without taking into account any particular
   characteristics or properties of individual blood samples.

   U.S. Patent Specification US-4,263,512 describes a known
   method and device for semi-quantitative sample quality
   monitoring of hemoglobin and bilirubin using multiple
15 wavelength measurements on diluted serum samples. This
   method has the following disadvantages:

       - it does not provide a quantitative determination of
   any interfering substances contained in the sample, and
       - it requires a special and specific conditioning of
20 the sample. Alternative methods are chromatographic or
   clinical-chemical determination of the concentrations of
   interfering substances. The chromatographic measurement
   requires a long measurement time and delicate
   instrumentation, whereas the clinical-chemical determination
25 is not suitable for reagentless measurement.

   Therefore, it is an aim of the present invention to provide
   a method for estimating rapidly the concentration of at
   least one interfering component in a fluid biological sample
   to be analyzed by an in vitro diagnostic test.

30 The above aim is attained with a method according to the
   invention as defined by claim 1. The remaining claims define
   preferred embodiments and applications thereof and means for
   carrying out the method.

   In a preferred method according to the invention, the
35 combination or superposition of the extinction spectrum of

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5   this first one of the components in a pure state and a
     function approximating the background extinction is fitted
     to the measured spectrum of the fluid to be analyzed in a
     wavelength range, where the component to be determined shows
     a significant or characteristic shape of its extinction
10   curve. The function approximating the background extinction
     may e.g. be a straight line, and in this case, the
     wavelength range is preferably chosen where the expected
     background extinction spectrum is similar to a straight
     line.

15   The invention shall be further explained in referring to
     exemplary embodiments with reference to the Figure:

     Fig. 1:   Schematic representation of a photometric
                      spectrum measurement arrangement.

     Fig. 2:   Extinction spectra of pure interfering
20   substances and a standard blood serum.

     Fig. 3:   Normalized extinction spectra of real whole
                      blood sera, bilirubin and hemoglobin
                      contribution being subtracted, and a reference
                      lipid solution sample.

25   Fig. 4:   Evaluation method for sample quality
                      monitoring.

     Fig. 5:   Experimentally measured extinction spectrum of
                      a real whole blood serum and results of the
                      evaluation method.

30   Fig. 6:   Measured extinction spectrum of a strongly
                      hemolytic whole blood sample and respective
                      extinction spectra obtained by the examination
                      method.

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5   Fig. 7:   As Fig. 6 for a strongly icteric whole blood
                   sample.

    Figs. 8, 9:   Optically determined hemoglobin (Fig. 8) and
                   bilirubin (Fig. 9) concentrations versus added
                   concentrations for 125 independent test
10   samples.

    Figs. 10,11:   Optically measured hemoglobin respectively
                   bilirubin concentration values vs. clinical-
                   chemically measured concentration values for
                   independent real whole blood sera.

15 Figs. 12-15:   Optically measured hemoglobin and bilirubin
                   concentrations of 92 real whole blood sera and
                   the respective CV values obtained using a
                   state-of-the-art spectrometer.

    Fig. 16, 17:   Low-cost versus state-of-the-art
20   spectroscopically measured (Fig. 16)
                   hemoglobin and bilirubin (Fig. 17)
                   concentrations of 92 real whole blood sera.

    Fig. 18:   Schematic illustration of a dip probe.

    A method according to the invention for sample quality
25 monitoring of blood serum or plasma by optical absorption
    spectroscopy in the visible and near IR range is described
    hereinafter. The target measuring ranges are 0.1 - 10 g/l
    hemoglobin, 2 - 20 mg/dl (1 dl = 0.1 liter) bilirubin and
    100 - 2000 mg/dl lipid with a measurement accuracy of 20 %.
30 The evaluation performed by the method according to the
    present invention yields e.g. the content of the hemoglobin
    and bilirubin. A quantitative determination of the lipid
    concentration by optical absorption spectroscopy is not
    possible due to a lack of a reproducible relation between

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5   light-scattering and lipid concentration. Therefore, a
    differential extinction spectrum is obtained by subtracting
    the hemoglobin and bilirubin contributions from the
    extinction spectrum of the target sample. It contains the
    spectral contributions of the lipid and the matrix, e.g. the
10 blood serum or plasma, which can then be investigated for
    spectral anomalies. The method has been experimentally
    tested using a series of 125 synthetic test samples and a
    series of 92 real blood sera. Accuracy and reproducibility
    of the technique versus the performance of the spectroscopic
15 measurement device are described and commented hereinafter.

    A basic setup for optical absorption spectroscopy for sample
    quality monitoring is shown in Fig. 1. A light beam 1
    emitted by a multiple optical wavelength light source 2 is
    collimated by a lens 3, which directs light of spectral
20 intensity $I_0(\lambda)$ to a target sample 6. The optical path
    within the target sample is denoted by d. A lens 4 collects
    transmitted light of intensity $I(\lambda)$, which is then detected
    by a spectral wavelength analyzer which has an input 5.

    Optical absorption is commonly characterized by the
25 extinction $E(\lambda)$, which is defined as

$$\frac{I(\lambda)}{I_0(\lambda)} = 10^{-E(\lambda)} . \qquad \Rightarrow \quad \log \frac{I(\lambda)}{I_0(\lambda)} = E(\lambda) \qquad (1)$$

    In the presence of J interfering substances in the target
    sample, e.g. hemoglobin, bilirubin and lipid (i. e., J=3),
    the extinction $E(\lambda)$ of the target sample can be described by
30 the linear combination

$$\overline{E}(\lambda) = \sum_{j=1}^{J} K_j(\lambda) \frac{d}{q_{dil}} C_j + E_g(\lambda) , \qquad (2)$$

5   where K_j and C_j are the specific extinction coefficient and
    the concentration of interfering substance j (j = 1, 2, …,
    J), respectively. The dilution of the target sample is
    denoted by q_{dil}, i. e. (original concentrations):(sample
    concentrations)=($1:q_{dil}$). E_g is the extinction characteristic

10 of the matrix, e.g. blood serum or plasma. In Figure 2 the
    extinction coefficients K_H of hemoglobin, K_B of bilirubin,
    and K_L of lipid (Intralipid 20% [Pharmacia, Sweden]) in the
    visible and near IR range are represented by lines 10, 11
    and 12 respectively. The extinction spectrum E_g of a

15 standard blood serum (Control Serum N (human) [Hoffmann-La
    Roche, Switzerland]) is represented in Fig. 2 by dashed line
    13.

    Within the scope of sample quality monitoring, a minimum
    number N_{min} = 4 of statistically independent extinction

20 values $E(\lambda_n)$ (n = 1, ..., 4) should allow the determination
    of the four unknown parameters in Eq. (2), i.e. the
    concentrations of the interference substances hemoglobin
    (C_H), bilirubin (C_B) and lipid (C_L), and the extinction
    component (E_g) corresponding to the matrix part. More

25 reproducible results are expected by least squares fitting
    the mathematical model of the extinction spectrum defined by
    Equation (2) to N > N_{min} measured values $E(\lambda_n)$ (n = 1, 2,
    ..., N) in order to obtain best estimates of the values of
    C_H, C_B and C_L.

30 However, it is observed that the specific extinction
    coefficient $K_L(\lambda)$ of lipid is not reproducible in real blood
    sera, and this is mainly due to the statistical distribution
    of the size of the scattering centers in the lipid.
    Moreover, as shown by Fig. 2, the monotonically decreasing

35 extinction spectrum of lipid versus wavelength lacks typical
    (local) characteristics. Therefore, the latter spectrum

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5   cannot be distinguished from the component (E_g) of the
     extinction spectrum which corresponds to the matrix.

     Figure 3 shows extinction spectra 31 of real whole blood
     sera, from which the hemoglobin and bilirubin contributions
     have been subtracted. These differential spectra 31
10   therefore represent the sum of the spectral contributions of
     the lipid and the matrix. The shown extinction spectra are
     normalized to the extinction at λ = 700 nm. The solid line
     30 represents the extinction spectrum of the reference
     solution of Intralipid, the broken lines 31 represent the
15   extinction spectra of several samples of real whole blood
     sera after subtraction of the extinction contributions of
     hemoglobin and bilirubin.

     Hence quantitative determination of the concentrations of
     hemoglobin (C_H), bilirubin (C_B) and lipid (C_L) does not
20   appear to be possible by measuring the optical extinction
     spectrum of the target sample and fitting the model in
     Equation (2) to the measured values $E(\lambda_n)$.

     Therefore, according to the invention, a sequential
     determination of first the hemoglobin (C_H) and then the
25   bilirubin (C_B) concentration is proposed in order to obtain
     a differential spectrum E_{diff} by subtracting the hemoglobin
     and bilirubin contributions from the measured extinction
     spectrum $E(\lambda)$. The differential spectrum E_{diff} thus
     represents· the sum of the contributions of the lipid (E_L)
30   and the matrix (E_g) to the extinction spectrum $E(\lambda)$ of the
     target sample, and may additionally be investigated for
     spectral anomalies over the whole spectral range.

     The bloc diagram in Fig. 4 summarizes an example of a
     proposed measurement and evaluation method according to the
35   invention for sample quality monitoring.

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5   This method is based on approximating the differential
spectrum $E_{diff} = E_g + E_L$ in a limited wavelength range λ_r by a
straight line.

In a first step 32, the hemoglobin concentration is
determined from a first measured extinction spectrum $E_1(\lambda)$

10   in a wavelength range $\lambda_{rh} \cong [545, 575]$ nanometer. A set of
extinction values comprising those of spectrum $E_1(\lambda)$ is
represented by block 35. As shown by Fig. 2, within the
wavelength range $\lambda_{rh} \cong [545, 575]$ nanometer hemoglobin has
typical spectral characteristics and the bilirubin

15   contribution to the extinction spectrum is quasi negligible
(Fig. 2).

In a curve fitting step 37, the mathematical model of the
extinction spectrum defined by Equations (3) and (4)
hereinafter is fitted by a linear least squares algorithm to

20   N_1 spectroscopically measured values $E_1(\lambda_n)$ (n = 1, 2, ...,
N_1) in the range λ_{rh}.

In curve fitting step 37, the extinction spectrum $E_1(\lambda_n)$ is
approximated by

$$\overline{E}_1(\lambda) = E_{d1}(\lambda) + E_H(\lambda) \tag{3}$$

25   where $E_H(\lambda)$ is the hemoglobin contribution and is defined by

$$E_H(\lambda) = K_H(\lambda)\frac{d}{q_{dil}}C_H \tag{4}$$

and E_{d1} linearly approximates the sum of the lipid and the
matrix contributions, and E_{d1} is a function defined by

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5    $E_{d1} = a_{0h} + a_{1h}\lambda$. Note that the parameters a_{0h} and a_{1h} have no physical significance.

Fitting step 37 delivers the best estimate of the values of the hemoglobin concentration C_H and a_{0h}, a_{1h}. This set of values is represented by block 38.

10   In a second step 33, the bilirubin concentration is determined from a second measured extinction spectrum $E_2(\lambda_n)$ in the wavelength range $\lambda_{rb} \equiv [480, 545]$ nanometer. A set of extinction values comprising those of spectrum $E_2(\lambda)$ is also represented by block 35.

15   In a curve fitting step 41, the mathematical model of the extinction spectrum defined by Equations (5) and (6) hereinafter is fitted by a linear least squares algorithm to N_2 spectroscopically measured values $E_2(\lambda_n)$ (n = 1, 2, ..., N_2) in the wavelength range λ_{rb}

20   In curve fitting step 41, the extinction spectrum $E_2(\lambda_n)$ is approximated by

$$\overline{E}_2(\lambda) = E_H(\lambda) + E_{d2}(\lambda) + E_B(\lambda) \tag{5}$$

where

$$E_B(\lambda) = K_B(\lambda)\frac{d}{q_{dil}}C_B \tag{6}$$

25   is the bilirubin contribution, E_H is the previously determined hemoglobin contribution, and E_{d2} linearly approximates the sum of the lipid and the matrix contributions and E_{d2} is a function defined in particular by $E_{d2} = a_{0b} + a_{1b}\lambda$, and in general by $E_{dk}(\lambda, a_{i,sk})$ with i

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5 ranging from 0 to at least 1. Note that the parameters a_{0b}
and a_{1b} have no physical significance.

Fitting step 41 delivers the best estimate of the bilirubin
concentration C_B and a_{0b}, a_{1b}. This set of values is
represented by block 42.

10 In a further method step 34, using Equations (2), (4) and
(6), the differential spectrum E_{diff}, , is obtained by the
equation

$$E_{diff}(\lambda) = E(\lambda) - E_H(\lambda) - E_B(\lambda) , \tag{7}$$

The set of values of E_{diff} is represented by block 45. This
15 set of values can then be investigated for spectral
anomalies and lead to results represented by block 46 which
can be in particular indicative of an anomalous lipid
content of the target sample.

The method according to the invention can be used to
20 determine the concentration of k pure substances contained
in a target sample.

For that purpose, in more general terms the method according
to the invention comprises:

    (a) measuring a first extinction spectrum $E_1(\lambda)$ of a
25 liquid sample in a first selected wavelength range
$\lambda= \lambda_{1,1}$ to $\lambda_{1,n}$, and

    (b) fitting an approximated spectrum $\overline{E}_1(\lambda)$ to said
first measured extinction spectrum $E_1(\lambda)$, said approximated
spectrum $\overline{E}_1(\lambda)$ being a combination of

30 a predetermined approximation function $E_{d1}(\lambda, a_{i,s1})$ for the
background extinction, with i ranging from zero to at least
one, and

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5   a predetermined extinction spectrum $E_{s1}(C_{s1}, \lambda)$ of a first pure component of concentration C_{s1} of the components to be determined,

said fitting being performed by varying said concentration C_{s1} of said first interfering component and at least two of

10   said coefficients $a_{i,s1}$, so that the deviation between said first measured extinction spectrum $E_1(\lambda)$ and said approximated spectrum $\overline{E}_1(\lambda)$ is minimized in order to determine the concentration of said first interfering component, and said first selected wavelength range being so

15   selected that the concentration C_{s1} of said first interfering component can be determined unambiguously.

In a preferred embodiment the approximated spectrum $\overline{E}_1(\lambda)$ is the sum of said predetermined approximation function $E_{d1}(\lambda, a_{i,s1})$ for the background extinction, and said

20   predetermined extinction spectrum $E_{s1}(C_{s1}, \lambda)$ of said pure first component of concentration C_{s1}.

In order to determine the concentration of a k pure component of a target sample a method according to the invention further comprises

25   (a) measuring at least one further extinction spectrum $E_k(\lambda)$ of said liquid sample in at least one further selected wavelength range $\lambda = \lambda_{k,1}$ to $\lambda_{k,n}$, with $k \geq 2$, and

        (b) fitting at least one further approximated spectrum $\overline{E}_k(\lambda)$ to said at least one further measured extinction

30   spectrum $E_k(\lambda)$, said at least one further approximated spectrum $\overline{E}_k(\lambda)$ being a combination of

a predetermined approximation function $E_{dk}(\lambda, a_{i,sk})$ for the background extinction, with i ranging from zero to at least one,

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previously determined extinction spectrums E_{SL} (C_{SL}, λ), with
L varying from L=1 to k-1, of k-1 pure components previously
determined, and

a predetermined extinction spectrum $E_{Sk}(C_{Sk}, \lambda)$ of a k pure
component of concentration C_{Sk} to be determined,
said fitting being performed by varying the concentration
C_{Sk} and at least two of the coefficients $a_{i,Sk}$ so that the
deviation between measured spectrum and approximated
spectrum is minimized, in order to determine the
concentration of the second component, said at least one
further selected wavelength range being so selected that the
concentration C_{Sk} of said k pure component can be determined
unambiguously.

In a preferred embodiment the approximated spectrum $\overline{E}_k(\lambda)$ is
the sum of said predetermined approximation function $E_{dk}(\lambda,$
$a_{i,Sk})$ for the background extinction, said previously
determined extinction spectrums E_{SL} (C_{SL}, λ), with L varying
from L=1 to k-1, of k-1 pure components previously
determined, and said predetermined extinction spectrum
$E_{Sk}(C_{Sk}, \lambda)$ of said k pure component of concentration C_{Sk}.

Reproducibility

The reproducibility of the measured concentration C_H of
hemoglobin and of the measured concentration C_B of bilirubin
can be analytically calculated, if the minimum number N_{min} =
3 of measured extinction values $E(\lambda_n)$ in the range λ_{rh} are
used to determine C_H from Equation (3) by means of the above
described fitting step 37, and if the minimum number N_{min} = 3
of measured extinction values $E(\lambda_n)$ in the range λ_{rb} are used
to determine C_B from Equation (5) by means of the above
described fitting step 41.

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5   The reproducibility of a measured concentration C is
    commonly characterized by the coefficient of variation CV =
    σ_c / $E\{c\}$, where σ_x and $E\{x\}$ stand for the standard deviation
    and the statistical expectation (mean value) of $\{x\}$,
    respectively. Using Equations (1), (2), and (7), it can be
10 readily shown that the coefficient of variation CV of the
    concentration C_j is related to the reproducibility of the
    (physically) measured optical intensity σ_I/I_0 by the relation

$$CV|_{N_{min}} = \frac{\sigma_{C_j}}{E\{C_j\}} \cong \frac{1}{D}\frac{\sigma_I}{I_0}\frac{1}{C_j} 10^{K_j(\bar{\lambda})\frac{d}{q_{dil}}C_j + E_d(\bar{\lambda})} \quad , \tag{8}$$

    where $\bar{\lambda}$ is the center wavelength of the respective
15 measurement range λ_r,

    $D = [\ln(10)/4]\cdot[2K_j(\lambda_2) - K_j(\lambda_1) - K_j(\lambda_3)]\cdot[d/q_{dil}]$,

    and $\ln(x)$ is the natural logarithm of (x).

    In Fig. 4, the center wavelength $\bar{\lambda}$ is pointed out by
    reference number 47.

20 Note that the background contribution $E_d(\lambda)$ of the lipid and
    the matrix significantly reduce the reproducibility of the
    measured concentration C_j.

    When $N > N_{min}$ statistically independent measured values $E(\lambda_n)$
    are used for the linear least squares algorithm, it can be
25 shown that the coefficient of variation CV of the measured
    hemoglobin concentration, as well as the coefficient of
    variation CV of the measured bilirubin concentration, is
    related to Equation (8) by the relation

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$$CV|_N = \frac{1}{\sqrt{M}} CV|_{N_{min}} , \qquad\qquad (9)$$

where $M = N - N_{min}$ is the number of redundant measurements.

In Fig. 4, a method step 48 for computing the coefficient of variation CV of the measured hemoglobin concentration, and a method step 49 for computing the coefficient of variation CV of the measured hemoglobin concentration are represented.

From Equation (9) it can be appreciated that the reproducibility of the measured concentration increases with the number N of measured extinction values considered for the curve fitting method step. The number N is given by the spectral resolution and sampling rate of the spectroscopic measurement system and the wavelength range λ_r.

It should be noted that an extension of the wavelength range λ_r increases N for a given spectral resolution and sampling rate, but that the linear approximation E_d of the sum of the lipid and the matrix contributions in Equations (3) and (5) becomes more and more inaccurate. The value of the coefficient of variation CV, calculated from Equations (8) and (9), can then be compared in a comparison step 50 with a predetermined limit value CV_{lim} in order to characterize the quality of the concentration measurement. If this comparison shows that a value of CV is larger than CV_{lim} this provides an indication 51 of a critical to weak reproducibility of the results, that is of the concentrations and the differential spectrum. Consequently, the measurement would e.g. be disregarded, repeated, or marked as being of reduced reliability.

Sample quality monitoring, based on optical absorption spectroscopy as shown in Fig. 1, has been experimentally

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investigated using a state-of-the-art spectrometer (Cary V, VARIAN, Australia). The collimated beam had an approximate spot size of 5 × 2 square millimeter. The optical path in the test sample was d = 10 mm. The sample dilution was 1:20 (q_{dil} = 20). The spectrum of the test sample was measured in the wavelength range λ = [300, 1200] nanometer with a spectral resolution of $\Delta\lambda$ = 0.05 nanometer and a spectral sampling rate of $\Delta\lambda_s$ = 1 nanometer/pixel. The hemoglobin concentration C_H has been obtained from linear least squares fitting the model in Equations (3) and (4) to N_1 = 28 measured values $E(\lambda_n)$ in the wavelength range λ_{rh} = [545, 575] nanometer. The bilirubin concentration C_B has been obtained from linear least squares fitting the model in Equations (5) and (6) to N_2 = 63 measured values $E(\lambda_n)$ in the wavelength range λ_{rb} = [480, 545] nanometer. The differential extinction spectrum E_{diff} has been obtained from Equation (7). The reproducibility of the measured hemoglobin and bilirubin concentrations has then been calculated according to Equations (8) and (9), with σ_I/I_0 = $5 \cdot 10^{-5}$ for the reproducibility of the measured optical intensities.

As an example, Figure 5 shows the experimentally measured extinction spectrum $E(\lambda)$ of a typical real whole blood serum 55. The best fitting extinction models for hemoglobin 57 and bilirubin 59 in Equations (3) and (5) are represented by crosses and dots, respectively. The best fitting hemoglobin and bilirubin concentrations are C_H = 0.18 g/l (CV = 1.5 %) and C_B = 0.67 mg/dl (CV = 0.3 %), respectively. The differential extinction spectrum $E_{diff}(\lambda_n)$ 60 is also shown by the dashed line.

Figs. 6 and 7 show other examples of real whole blood serum samples, namely with a high hemoglobin content respectively

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5   an highly icteric sample. Furthermore, in Fig. 6, the
differential spectrum shows an anomalous differential
spectrum which is merely constant with additionally an
increased extinction with increasing wavelength above about
650 nanometer. The continuous line 62 is the measured
10   spectrum, the dashed line 64 and the dotted line 65 are the
hemoglobin respectively the bilirubin contributions, and the
dash-dotted line 66 is the differential spectrum, each time
calculated from the results according to the described
method.

15   **Accuracy**

In order to investigate the accuracy of the method, the
hemoglobin and bilirubin concentrations of a series of 125
independent test samples have been determined. The samples
have been synthesized using a standard blood serum (Control
20   Serum N (human) [Hoffmann-La Roche, Switzerland]) to which
hemoglobin (Hemolysat [Hoffmann-La Roche, Switzerland]),
bilirubin (B-4126 mixed isomers [Sigma, Switzerland]) and
lipid (Intralipid 20% [Pharmacia, Sweden]) have been added.
The added concentrations of hemoglobin, bilirubin and lipid
25   were C_H = [0, 0.17, 0.83, 3.33, 15] g/l, C_B = [0, 1, 2, 10,
20] mg/dl and C_L = [0, 50, 100, 400, 1800] mg/dl,
respectively, leading to the set of 5·5·5 test samples. The
optically measured hemoglobin 70 and bilirubin 72
concentrations versus added concentrations are represented
30   in Fig. 8 and 9.

In the case of hemoglobin (Fig. 8), a linear least squares
fit 74 ($c_{fit,h}$ = $c_{0,h}$ + $m_h C_H$) yields an offset concentration
$c_{0,h}$ = 0.12 g/l and a slope m_h = 0.95. The correlation
coefficient between the best fit and the measured values is
35   ρ = 0.999. In the case of bilirubin (Fig. 9), a second
linear least squares fit 76 ($c_{fit,b}$ = $c_{0,b}$ + $m_b C_B$) yields an

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5   offset concentration $c_{0,b}$ = 1.64 mg/dl and a slope m_b = 0.999. The correlation coefficient between the best fit and the measured values is ρ = 0.995. Note that Control Serum N (human) has an approximate bilirubin concentration of $C_B \cong 2$ mg/dl. Further, it is stated that the amount of added

10   hemoglobin, bilirubin and lipid also has finite accuracy.

The hemoglobin and bilirubin concentrations of a series of 92 real whole blood sera have then been optically determined. The concentration values were in the range C_H = [0, 5] g/l for hemoglobin and C_B = [0, 45] mg/dl for

15   bilirubin. As reference values, the concentrations have been determined by clinical-chemical analysis (Cobas® Integra 700 analyzer, [Hoffmann-La Roche, Switzerland]). Figures 10 and 11 show the optically versus clinical-chemically determined hemoglobin [bilirubin] concentrations 90 [91].

20   The results show that the sensitivity of the method is approximately $C_{H,min} \cong 0.5$ g/l hemoglobin and $C_{B,min} \cong 2$ mg/dl bilirubin. The observed correlation coefficients between the reference 93 [94] and the optically determined 90 [91] hemoglobin [bilirubin] concentrations were ρ = 0.980 and ρ =

25   0.996, respectively. Note that the clinical-chemical method has also limited accuracy; namely the bilirubin concentrations (Fig. 11) show better correlation than the hemoglobin concentrations (Fig. 10), although the accuracy of the optically measured bilirubin concentration is

30   affected by the accuracy of the hemoglobin concentration determination (sequential determination of hemoglobin and bilirubin, see above).

In comparison, the benchmark Hitachi-Formula (US-4,263,512) evaluation algorithm has been used to evaluate the optical

35   absorption spectra. The observed correlation coefficients

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5   between the reference and the Hitachi-Formula determined
concentration values were $\rho = 0.879$ for hemoglobin and $\rho = 0.992$ for bilirubin.

Reproducibility

The coefficient of variation CV of the measured hemoglobin
10 and bilirubin concentrations has been calculated from
Equation (8), where $\sigma_I/I_0 = 5 \cdot 10^{-5}$ was the reproducibility of
the measured optical intensities and $\bar{\lambda} = 560\,nm$ was the
center wavelength of λ_{rh}. for hemoglobin and $\bar{\lambda} = 512\,nm$ for
bilirubin was the center wavelength of λ_b. Figures 12 to 15
15 show the optically measured hemoglobin (Fig. 12) and
bilirubin (Fig. 14) concentrations of the set of 92 real
blood sera and the respective CV values (Fig. 13
respectively Fig. 15). Inspection of Figs. 12 to 15 shows
that the reproducibility is better for large concentration
20 values, and that the values for hemoglobin and bilirubin are
better than CV < 10 % respectively < 1 % for 89 respectively
91 of 92 analyzed sera.

Low cost optical spectrometer setup

Sample quality monitoring, based on optical absorption
25 spectroscopy as shown in Fig. 1, has then been
experimentally investigated with low cost optical elements.
The multiple optical wavelength light source was a white-
light halogen lamp (Halogen 5V, 5W, $P_v \cong 2$ nW/nanometer @ λ
= 530 nanometer [MICROPARTS GmbH, Germany]). The collimated
30 beam had an approximate diameter of D = 2 mm. The optical
path in the test sample was d = 10 mm. The dilution of the
sample was 1 : 20 ($q_{dil} = 20$). The transmitted light was
collected by a lens (focal length f = 5 mm) and coupled into
an optical fiber with core diameter $\varnothing_c = 100$ μm. The light

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5 was spectroscopically analyzed by a low cost, plane-concave
spectrometer PCS [CSEM-Z, Switzerland] with spectral
resolution $\Delta\lambda \cong 8$ nanometer. The spectrum of the test sample
was measured by a linear photodiode array (512 pixels,
center-to-center spacing $\Delta x = 25$ μm) in the wavelength range

10 $\lambda = [421, 704]$ nanometer. The spectral sampling rate was $\Delta\lambda_s$
$= 2.8$ nanometer/pixel. The reproducibility of the measured
optical intensities was $\sigma_I/I_0 = 5 \cdot 10^{-4}$. The hemoglobin
concentration C_H has been obtained from linear least squares
fitting the model in Equations (3) and (4) to $N_1 = 11$

15 measured values $E(\lambda_n)$ in the wavelength range $\lambda_{rh} = [545,
575]$ nanometer. The bilirubin concentration C_B has been
obtained from linear least squares fitting the model in
Equations (5) and (6) to $N_2 = 20$ measured values $E(\lambda_n)$ in the
wavelength range $\lambda_{rb} = [480, 545]$ nanometer. The differential

20 extinction spectrum E_{diff} has been obtained from Equation
(7). Figures 16 and 17 show the PCS versus the state-of-the-
art (Cary V) spectroscopically measured hemoglobin
respectively bilirubin concentrations of the set of 92 blood
sera of Figs. 10, 11 and 12 to 15. In the case of

25 hemoglobin (Fig. 16), a linear least squares fit ($c_{fit,h} = c_{0,h}$
$+ m_h C_H$) in the concentration range $C_H < 2$ g/l yields an
offset concentration $c_{0,h} = 0.043$ g/l and a slope $m_h = 0.859$.
The correlation coefficient between the best fitting curve
and the PCS measured values is $\rho = 0.997$. In the case of

30 bilirubin (Fig. 17), a linear least squares fit ($c_{fit,b} = c_{0,b}$
$+ m_b C_B$) in the concentration range $C_B < 15$ mg/dl yields an
offset concentration $c_{0,b} = -0.010$ mg/dl and a slope $m_b =
0.940$. The correlation coefficient between the best fitting
curve and the PCS measured values is $\rho = 0.998$. The results

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5   show that low cost spectrometers can readily be used for
       sample quality monitoring purposes.

       If the examination of the samples yields a result indicating
       an anomalous condition of the sample, there may be generated
       by the examining device, e. g., one or more of the following
10   signals or responses:

       - an optical and/or acoustical warning signal to excite the
         operator's attention, particularly in case an abnormal
         sample has been detected,

       - a printout of results (spectra, coefficients etc.) on a
15   printer,

       - a print on the analyzer's printout, so that the operator
         can immediately see if the results of the regular,
         chemical-clinical examination are true or prone to
         artefacts, or

20   - an automatic repetition of the measurement, e.g. using a
         new test sample.

       The described method may be implemented in various
       arrangements, preferably in connection with an automated
       analyzer, e g. as follows:

25   - The quality test may be done as a first photometric pass
         in the photometric site of an analyzer. Thereby, the
         performance of the analyzer is reduced because this
         prescan and the regular photometric pass are performed
         subsequently, or an additional sample is needed causing
30   consumption of sample material;

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5   — An additional photometric site is provided for the
     quality test;

     — The pipette, or more generally, the supply system of the
     analyzer for the fluids to be tested, is provided with a
     transparent site, i. e. an optical flow-through cell
10   (OFTC), in connection with a photometer; where necessary,
     particularly when the conduit system subsequently
     provides differently diluted samples, there may be
     arranged different OFTC paths with different optical path
     lengths in connection with flow switches for compensating
15   the varying dilutions;

     — A stand-alone photometer dedicated to the quality tests

     — A dip-in probe 110 having e.g. a structure of the type
     shown by Fig. 18. Probe 110 has a tubular body 111 and is
     adapted to be immersed into a liquid sample contained in
20   a sample container. Light guides 115 and 119 are arranged
     within and extend along the length axis of tubular body
     111. Tubular body 111 has an end part 112 and a recess
     113 spaced from end part 112. Tubular body has a segment
     114 of reduced cross-section. The position of this
25   segment corresponds to the position of recess 113. Lenses
     117 and 118, and a prism 116 arranged within tubular body
     111 at the end 112 thereof complete the structure of dip-
     in probe 110. Light transmitted through light guide 115
     and lens 118 impinges on and is reflected back by prism
30   116, traverses recess 113, where a volume of the liquid
     sample to be examined is present, is collected by lens
     117 and is transmitted to a photometer via light guide
     119.

     — The sample tube itself may be used as the photometric
35   cuvette, provided the differing lengths of the optical

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5   observation paths can be compensated for, i. e. the path
       lengths are determined and can be input into the quality
       test system, and/or the sample tubes are of sufficiently
       equal size so that the optical paths do only differ
       within small limits, maybe in an even negligible
10   variation range.

From the above description, variations of the invention are
conceivable to the one skilled in the art without leaving
the scope of the invention as defined in the claims. For
instance, it is conceivable:

15   — to extend the method to the determination of a third and
         further components by continuing the sequential
         determination method using two, three, four etc.
         previously determined components for ascertaining the
         concentrations of a third, fourth etc. component;

20   — to have the differential spectrum analyzed automatically
         by determining its curvature (i. e. the second
         derivative) and/or slope (i. e. the first derivative),
         which should increase respectively be negative for
         increasing wavelength in the exemplary quality test set
25   forth above;

       — to choose deviating wavelength ranges for the photometric
         measurements, particularly if the quality test is used
         for determining other components of the samples provided
         that the spectra to be combined in order to approximate
30   the measured spectrum show peculiarities in the given
         wavelength range so that the approximation parameters,
         before all the concentration of the sought component, are
         unambiguously derivable;

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5   — to determine the differential spectrum in a subrange of
the wavelength range used for the determination of the
single components, or possibly even a range extending
beyond this range.

List of reference numbers

10   1   beam of light

     2   light source (multiple wavelenght)

     3   lens

     4   lens

     5   input of wavelength analyzer

15   10   extinction coefficient K_H of hemoglobin

     11   extinction coefficient K_H of bilirubin

     12   extinction coefficient K_L of lipid

     13   extinction coefficient E_g of a standard blood serum

     30   extinction spectra of the reference solution of
20   Intralipid

     31   extinction spectra of several samples of real whole
            blood sera

     32   method step: determination of hemoglobin concentration

     33   method step: determination of bilirubin concentration

25   34   method step: determination of E_{diff} and further
     evaluation

     35   measured extinction spectrum $E(\lambda)$ 35 in the
            approximate wavelength range $\lambda_{rh} \cong$ [545, 575] nm

     36   determination of E_{d1} at $\bar{\lambda}$ = 560 nm

30   37   method step: curve fitting

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5 38   set of values: C_H and a_{0h}, a_{1h}

   40   determination of E_{d1} at $\bar{\lambda} = 512\,nm$

   41   method step: curve fitting

   42   set of values: C_B and a_{0b}, a_{1b}

   45   determination of E_{diff}

10 46   method step: investigation for spectral anomalies 46

   47   center wavelength

   48   computation of the coefficient of variation CV of the
           measured hemoglobin concentration

   49   computation of the coefficient of variation CV of the
15   measured bilirubin concentration

   50   comparison step

   51   indication of a critical to weak reproducibility of
           the results

   55   measured extinction spectrum $E(\lambda)$ of a typical real
20   whole blood serum

   57   best fitting extinction model for hemoglobin

   59   best fitting extinction models for bilirubin

   60   differential extinction spectrum $E_{diff}(\lambda_n)$

   62   measured spectrum

25 64   hemoglobin contribution

   65   bilirubin contribution

   66   differential spectrum

   70   optically measured hemoglobin concentration

   72   optically measured bilirubin concentration

30 74   linear least squares fit

5 76   linear least squares fit

   90   optically determined hemoglobin concentrations

   91   optically determined bilirubin concentrations

   93   clinical-chemically determined hemoglobin
          concentrations

10 94   clinical-chemically determined bilirubin
          concentrations

   96   state-of-the-art (Cary V) spectroscopically measured
          hemoglobin concentrations

   97   linear least squares fit

15 99   state-of-the-art (Cary V) spectroscopically measured
          bilirubin concentrations

   100   linear least squares fit

   110   dip-in probe

   111   tubular body

20 112   end part of tubular body 111

   113   recess

   114   segment having a reduced cross-section

   115   light guide

   116   prism

25 117   lens

   118   lens

   119   light guide